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L13: Entry 3 of 5

File: USPT

May 4, 2004

US-PAT-NO: 6730777

DOCUMENT-IDENTIFIER: US 6730777 B1

TITLE: Cystic fibrosis gene

DATE-ISSUED: May 4, 2004

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Tsui; Lap-Chee	Toronto			CA
Riordan; John R.	Toronto			CA
Collins; Francis S.	Ann Arbor	MI		
Rommens; Johanna M.	Willowdale			CA
Iannuzzi; Michael C.	Ann Arbor	MI		
Kerem; Bat-Sheva	Toronto			CA
Drumm; Mitchell L.	Ann Arbor	MI		
Buchwald; Manuel	Toronto			CA

US-CL-CURRENT: 530/350

## CLAIMS:

We claim:

1. An isolated polypeptide comprising an amino acid sequence (a) according to FIGS. 1A-1H for amino acid residue positions from 1 to 1480 comprising a normal CFTR polypeptide.

2. A polypeptide according to claim 1 made by chemical or enzymatic peptide synthesis.

3. An isolated mutant CFTR polypeptide, comprising at least one CF mutation of a normal CFTR polypeptide having the sequence according to FIGS. 1A-1H for amino acid residue positions 1 to 1480, wherein said CF mutation includes the deletion of phenylalanine from amino acid residue position 508 of FIGS. 1A-1H.

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L5: Entry 12 of 13

File: USPT

Apr 19, 2005

DOCUMENT-IDENTIFIER: US 6881825 B1

TITLE: Identification of peptides that facilitate uptake and cytoplasmic and/or nuclear transport of proteins, DNA and viruses

Detailed Description Text (61):

In accordance with the present invention, screening for internalizing peptides by phage biopanning yielded the peptides represented by SEQ ID NO:1 through SEQ ID NO:18, further illustrated in Table 1 above. Particularly preferred peptides include KRIHPRLTRSIR (SEQ ID NO:2), PPRLRKRRQLNM (SEQ ID NO:3), PIRRRKKLRLK (SEQ ID NO:4) and RRQRRTSKLMKR (SEQ ID NO:5) which facilitated the internalization of phage as well as the facilitation of the internalization of a cargo (e.g. .beta.-gal and Cy3).

Detailed Description Paragraph Table (1):

TABLE 1 1) peptide 1 (pep1) KRIIQRILSRNS (SEQ ID NO:1) 2) peptide 2 (pep2) KRIHPRLTRSIR (SEQ ID NO:2) 3) peptide 3 (pep3) PPRLRKRRQLNM (SEQ ID NO:3) 4) peptide 4 (pep4) PIRRRKKLRLK (SEQ ID NO:4) 5) peptide 5 (pep5) RRQRRTSKLMKR (SEQ ID NO:5) 6) peptide 6 (pep6) MHKRPTTPSRKM (SEQ ID NO:6) 7) peptide 7 (pep7) RQSRRRRPLNIR (SEQ ID NO:7) 8) peptide 8 (pep8) RIRMIQNLIKKT (SEQ ID NO:8) 9) peptide 9 (pep9) SRRKRQRSNMRI (SEQ ID NO:9) 10) peptide 10 (pep10) QRIRKSKISRTL (SEQ ID NO:10) 11) peptide 11 (pep11) PSKRLHLNNLRR (SEQ ID NO:11) 12) peptide 12 (pep12) HRHIRRQSLIML (SEQ ID NO:12) 13) peptide 13 (pep13) PQNRLQIRRHSH (SEQ ID NO:13) 14) peptide 14 (pep14) PPHNRIQRRLNM (SEQ ID NO:14) 15) peptide 15 (pep15) SMLKRHNHSTSNR (SEQ ID NO:15) 16) peptide 16 (pep16) GSRHPSLIIPRQ (SEQ ID NO:16) 17) peptide 17 (pep17) SPMQKTMNLPPM (SEQ ID NO:17) 18) peptide 18 (pep18) NKRILIRIMTRP (SEQ ID NO:18) 19) peptide 19 (pep19) HGWZIHGLLHRA (SEQ ID NO:25) 20) peptide 20 (pep20) AVPAKKRZKSV (SEQ ID NO:26) 21) peptide 21 (pep21) PNTRVRPDVSF (SEQ ID NO:27) 22) peptide 22 (pep22) LTRNYEAWVPTP (SEQ ID NO:28) 23) peptide 23 (pep23) SAETVESCLAKSH (SEQ ID NO:29) 24) peptide 24 (pep24) QSHIATLPFTPT (SEQ ID NO:30) 25) peptide 25 (pep25) SYIQRTPTSTLP (SEQ ID NO:31) 26) peptide 26 (pep26) AVPAENALNNPF (SEQ ID NO:32) 27) peptide 27 (pep27) SFHQFARATLAS (SEQ ID NO:33) 28) peptide 28 (pep28) QSPTDFTFPNPL (SEQ ID NO:34) 29) peptide 29 (pep29) HFAAWGGWSLVH (SEQ ID NO:35) 30) peptide 30 (pep30) HIQLSPFSQSWR (SEQ ID NO:36) 31) peptide 31 (pep31) LTMPSDLQPVLW (SEQ ID NO:37)

CLAIMS:

1. A peptide having a amino acid sequence selected from the group consisting of PIRRRKKLRLK (SEQ ID NO:4); RRQRRTSKLMKR (SEQ ID NO:5); SRRKRQRSNMRI (SEQ ID NO:9); SFHQFARATLAS (SEQ ID NO:33); DPATNPGPHFPR (SEQ ID NO:58); and TLPSPALLTVH (SEQ ID NO:59).

4. The peptide of claim 2 wherein the peptide is RRQRRTSKLMKR (SEQ ID NO:5).

10. A method for inducing synovial cell death comprising administering a peptide-cargo complex to said synovial cell wherein the peptide has an amino acid sequence selected from the group consisting of PIRRRKKLRLK (SEQ ID NO:4), RRQRRTSKLMKR (SEQ ID NO:5), SRRKRQRSNMRI (SEQ ID NO:9), SFHQFARATLAS (SEQ ID NO:33), DPATNPGPHFPR (SEQ ID NO:58) and TLPSPALLTVH (SEQ ID NO:59).

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L7: Entry 11 of 11

File: USPT

Apr 19, 2005

DOCUMENT-IDENTIFIER: US 6881825 B1

TITLE: Identification of peptides that facilitate uptake and cytoplasmic and/or nuclear transport of proteins, DNA and viruses

Detailed Description Text (18):

Additionally, the ability of the internalizing peptide to carry the cargo into the cell may be measured by the presence of functional cargo in the cell (e.g. the presence of .beta.-gal may be demonstrated by the ability of the cell to cleave X-gal and give a blue color; the presence of cystic fibrosis transmembrane regulator (CFTR) protein may be demonstrated by the presence of a functional chloride ion channel in a cell originally lacking CFTR, and the presence of an apoptotic factor may be shown by the apoptosis of cells after the administration of a peptide-apoptosis factor construct of the present invention). The cargo (e.g. polypeptide, polynucleotide, small molecule, virus, plasmid) may be labeled by a method known in the art (e.g. radiolabeling or fluorescent labeling) and the presence of the label would establish the efficient delivery of the cargo into the target cell by the internalizing peptide. In addition, the presence of an immunogen in the cell of a subject may be measured by the ability to elicit an immune response in a subject.

Detailed Description Text (60):

The peptide conjugates (peptide+cargo) are then incubated with a target cell to allow for delivery of the peptide-cargo complex into the cell (e.g. Hig-82 cells). The ability of the peptide to transfer the cargo into the target cell may be measured by the presence of the cargo in the target cell by techniques known in the art. Where the cargo is .beta.-gal, the addition of Xgal to the cells will produce a blue color in the cells if the .beta.-gal is present. Where the cargo is Cy3, confocal microscopy may be employed to determine whether the cells fluoresce. Functional assays may also determine the presence of cargo in a cell. For example, but not by way of limitation, where the cargo is CFTR (or a nucleic acid encoding CFTR), the manifestation of a functional chloride ion channel would indicate delivery of the CFTR cargo to the target cell. Where the cargo is a toxin, cell death may indicate the presence of the cargo in the target cell and, where the cargo is a virus (e.g. Human Immunodeficiency Virus, Murine Leukemia Virus, Equine Infections Anemia Virus), the virus may comprise green fluorescent protein (GFP) as a marker or the virus may be labeled with Cy3, also a fluorescent marker to track the internalization of the virus by the peptides of the present invention in cells which would otherwise be resistant to infection by the virus. If the virus is a viral vector comprising a transgene, the presence of the virus in the cell may be demonstrated by the presence of a transgene product. The presence of the cargo in the nuclei by the methods described above, indicates that the peptides are capable of facilitating the translocation of the cargo to the nucleus and may be demonstrated as described for internalization generally. For example, confocal microscopy may be used to demonstrate the presence of a fluorescent tagged molecule in the nucleus. Alternatively, the cells may be harvested and the nuclei separated therefrom for the determination of the presence of a functional cargo therein by methods known to those skilled in the art.

Detailed Description Text (108):

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L12: Entry 1 of 1

File: USPT

Jun 16, 1998

US-PAT-NO: 5767084DOCUMENT-IDENTIFIER: US 5767084 A

TITLE: Method of treatment for cystic fibrosis and peptides for same

DATE-ISSUED: June 16, 1998

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Price; Elmer M.	Hartsburg	MO		
Clarke; Lane L.	Columbia	MO		

US-CL-CURRENT: 514/16; 514/18, 514/2, 514/851, 530/300, 530/329, 530/330

## CLAIMS:

We claim:

1. A method of inhibiting degradation and/or retention of mutant CFTR protein by administering a peptide consisting of the amino acid sequence Asn-Ile-Ile-Gly-Val-Ser-Tyr (SEQ ID No: 2), optionally acetylated at its amino terminus.
2. A method of preventing cellular retention and degradation of otherwise membrane bound mutant CFTR proteins by competitively inhibiting degradation and retention which would otherwise retain or degrade newly synthesized mutant CFTR proteins prior to arrival of the mutant CFTR proteins at the cell membrane by administering a peptide consisting of the amino acid sequence Asn-Ile-Ile-Gly-Val-Ser-Tyr (SEQ ID No: 2), optionally acetylated at its amino terminus.
3. A peptide consisting of the amino acid sequence Asn-Ile-Ile-Gly-Val-Ser-Tyr (SEQ ID No: 2), optionally acetylated at its amino terminus.

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L13: Entry 1 of 5

File: USPT

Jan 10, 2006

US-PAT-NO: 6984487

DOCUMENT-IDENTIFIER: US 6984487 B1

TITLE: Cystic fibrosis gene

DATE-ISSUED: January 10, 2006

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Tsui; Lap-Chee	Toronto			CA
Riordan; John R.	Toronto			CA
Collins; Francis S.	Ann Arbor	MI		US
Rommens; Johanna M.	Willowdale			CA
Iannuzzi; Michael C.	Ann Arbor	MI		US
Kerem; Bat-Sheva	Toronto			CA
Drumm; Mitchell L.	Ann Arbor	MI		US
Buchwald; Manuel	Toronto			CA

US-CL-CURRENT: 435/6; 530/350, 536/23.1

## CLAIMS:

We claim:

1. A purified DNA molecule, comprising a cystic fibrosis transmembrane conductance regulator (CFTR) DNA sequence selected from the group consisting of: (a) a DNA sequence encoding a normal CFTR protein having the amino acid sequence depicted in FIG. 1; (b) a DNA sequence which hybridizes under stringent conditions to at least 16 contiguous nucleotides of the DNA sequence depicted in FIG. 1; and (c) a DNA sequence complementary to the DNA sequence of (a) or (b), wherein said DNA sequence of (a), (b) or (c), when present as part of a coding sequence of a normal CFTR gene, is expressed in human epithelial cells as a normal CFTR protein which is not characterized as having cystic fibrosis associated activity.
2. A purified normal CFTR RNA molecule, comprising an RNA sequence having the DNA sequence recited in claim 1, wherein, in said RNA sequence, each thymidylate in said DNA sequence is replaced with a uridine.
3. A purified DNA molecule according to claim 1, wherein said purified DNA molecule is cDNA.
4. A purified DNA molecule according to claim 1, comprising a DNA sequence encoding an amino acid sequence depicted in FIG. 1 selected from the group consisting of amino acid positions: (a) 28 to 45; (b) 58 to 75; (c) 104 to 117; (d) 139 to 153; (e) 204 to 249; (f) 279 to 294; (g) 347 to 698; (h) 500

to 512; (i) 710 to 757 (j) 725 to 739; (k) 758 to 796; (l) 933 to 946; (m) 1066 to 1084; and (n) 1188 to 1480.

5. A detectably labeled normal CFTR probe, comprising a purified DNA or RNA nucleotide sequence, said DNA or RNA nucleotide sequence having at least 16 contiguous nucleotides being the same as, or complementary to, a DNA sequence according to claim 1, wherein, in said RNA nucleotide sequence, each thymidylate in said DNA nucleotide sequence is replaced with a uridine, and wherein said probe is detectably labeled and distinguishes a normal CFTR gene from a mutant CFTR gene.

6. A normal CFTR probe according to claim 5, wherein (i) said purified DNA sequence comprises AAA GAA AAT ATC ATC TTT GGT GTT, or a complementary sequence thereof; or (ii) said purified RNA sequence comprises AAA GAA AAU AUC AUC UUU GGU GUU, or a complementary sequence thereof.

7. A purified DNA molecule, comprising a mutant CFTR DNA sequence selected from the group consisting of: (a) a DNA sequence encoding a mutant CFTR protein having the amino acid sequence depicted in FIG. 1, wherein the mutation is the .DELTA.F508 mutation which comprises a three base pair deletion which results in the deletion of the DNA sequence encoding phenylalanine at amino acid position 508 of a CFTR protein as depicted in FIG. 1; (b) a DNA sequence which hybridizes under stringent conditions to at least 16 contiguous nucleotides of the DNA sequence of (a), said DNA sequence containing the .DELTA.F508 CF mutation; and (c) a DNA sequence complementary to the DNA sequence of (a) or (b); wherein said DNA sequence of (a), (b) or (c), when present as part of a coding sequence of said mutant CFTR gene, is expressed in human epithelial cells which is characterized as having cystic fibrosis associated activity.

8. A purified mutant CFTR RNA molecule, comprising an RNA sequence having the DNA sequence of claim 7, wherein, in said RNA sequence, each thymidylate in said DNA sequence is replaced with a uridine.

9. A purified DNA molecule according to claim 7, wherein said purified DNA is cDNA.

10. A detectably labeled CFTR probe, comprising a purified DNA or RNA nucleotide sequence, said DNA or RNA nucleotide sequence having at least 16 contiguous nucleotides being the same as, or complementary to, the DNA sequence of claim 7, wherein, in said RNA nucleotide sequence, each thymidylate in said DNA nucleotide sequence is replaced with a uridine, and wherein said probe is detectably labeled and distinguishes the .DELTA.F508 mutant CFTR gene from a normal CFTR gene.

11. A CFTR probe according to claim 10, wherein (i) said purified DNA sequence comprises AAA GAA AAT ATC ATT GGT GTT, or a complementary sequence thereof; or (ii) said purified RNA sequence comprises AAA GAA AAU AUC AUU GGU GUU, or a complementary sequence thereof.

12. A method for screening a subject to determine if said subject is a CF carrier or a CF patient, comprising: (a) providing a biological sample of said subject to be screened; and (b) assaying the biological sample for the presence of the CFTR DNA sequence of claim 1, or a mutant CFTR DNA sequence of claim 7, wherein said assay is a DNA screening assay which detects normal or the .DELTA.F508 mutant CFTR DNA sequences by at least one assay method selected from the group consisting of probe hybridization, direct DNA sequencing, restriction enzyme analysis, electrophoretic mobility, RNase

protection, chemical cleavage and ligase-mediated detection.

13. A method according to claim 12, wherein the biological sample includes at least part of the genome of the subject comprising the 7q31 region of chromosome 7.

14. A method according to claim 12, wherein said assay method is probe hybridization, comprising: (a) isolating and optionally amplifying genomic DNA from said biological sample; (b) hybridizing a first detectably labeled CFTR specific DNA or RNA probe to said isolated genomic or amplified DNA, said first DNA or RNA probe stringently hybridizing to at least 16 contiguous nucleotides of a DNA sequence depicted in FIG. 1, a sequence complementary thereto, or a sequence thereof further having at least the .DELTA.F508 cystic fibrosis (CF) mutation; and (c) treating said genomic DNA to determine the presence or absence of the detectably labeled DNA or RNA first probe hybridized to said genomic DNA and thereby indicating, in accordance with a predetermined manner of hybridization, the presence or absence of said CF mutation.

15. A method according to claim 14, wherein the labeled nucleotide probe is a probe according to claim 5.

16. A method according to claim 14, wherein the labeled nucleotide probe is a probe according to claim 10.

17. A method according to claim 14, wherein said probe comprises a nucleotide sequence according to claim 6.

18. A method according to claim 14, wherein said probe comprises a nucleotide sequence according to claim 11.

19. A method according to claim 14, wherein said subject is a human.

20. A method according to claim 19, wherein said human is a human fetus in utero.

21. A method according to claim 14, wherein the assay further includes at least one additional probe as said detectably labeled CFTR specific DNA or RNA probe.

22. A method according to claim 14, wherein the assay further includes hybridizing to said isolated genomic DNA a second detectably labeled, CFTR specific, nucleotide DNA or RNA probe having a DNA or RNA sequence that hybridizes to at least 16 contiguous nucleotides of the DNA of FIG. 1 or a sequence complementary thereto, said second probe having a nucleotide sequence different from said first probe.

23. A method according to claim 14, wherein said assay method is restriction enzyme analysis, comprising determining the presence or absence of a restriction endonuclease site in said mutant CFTR gene, wherein said presence or absence of the restriction site corresponds to the presence of at least said cystic fibrosis (CF) mutation.

24. A method according to claim 12, wherein said assay method is electrophoretic mobility, comprising determining differential mobility of heteroduplex polymerase chain reaction (PCR) products in polyacrylamide gels as a result of deletions of said CFTR DNA sequence, wherein the differential

mobility corresponds to the presence or absence of at least said CF mutation in a CFTR DNA sequence, or an RNA homologue thereof.

25. A kit for assaying for the presence of a normal CFTR gene, comprising in separate containers: (a) at least one oligonucleotide probe comprising a detectably labeled normal CFTR probe(s) according to claim 5, for hybridizing specifically to a corresponding portion of a normal CFTR gene; (b) a device in which a biological sample containing genomic or amplified DNA or RNA and said probe(s) are to be combined; (c) a reagent element in association with said probe(s) to indicate hybridization of said probe(s) to said normal CFTR gene or PCR amplified portion thereof.

26. A kit for assaying for the presence of the .DELTA.F508 mutant CFTR gene, comprising in separate containers: (a) at least one oligonucleotide probe comprising a detectably labeled mutant CFTR probe(s) according to claim 10, for hybridizing specifically to a corresponding portion of said mutant CF gene; (b) a device in which a biological sample containing genomic or amplified CFTR DNA or RNA and said probe(s) are to be combined; (c) a reagent element in association with said probe(s) to indicate hybridization of said probe(s) to said mutant CFTR gene or PCR amplified portion thereof.

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File: USPT

Jul 7, 1998

US-PAT-NO: 5776677

DOCUMENT-IDENTIFIER: US 5776677 A

TITLE: Methods of detecting cystic fibrosis gene by nucleic acid hybridization

DATE-ISSUED: July 7, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Tsui; Lap-Chee	Toronto			CA
Riordan; John R.	Toronto			CA
Collins; Francis S.	Ann Arbor	MI		
Rommens; Johanna M.	Willowdale			CA
Iannuzzi; Michael C.	Ann Arbor	MI		
Kerem; Bat-Sheva	Toronto			CA
Drumm; Mitchell L.	Ann Arbor	MI		
Buchwald; Manuel	Toronto			CA

US-CL-CURRENT: [435/6](#); [435/91.2](#), [536/23.2](#), [536/24.3](#), [536/24.33](#)

CLAIMS:

We claim:

1. A method for screening a subject to determine if said subject is a CF carrier or a CF patient, comprising:

(a) providing a biological sample of the subject to be screened, said sample containing a mutant or a normal CFTR gene; and

(b) assaying said biological sample for the mutant or the normal CFTR gene, wherein the assay includes:

(i) assaying for the presence of a normal CFTR gene by hybridization comprising:

(A) an oligonucleotide probe which specifically binds to a normal DNA molecule encoding a normal CFTR polypeptide, wherein the normal DNA molecule comprises a DNA sequence selected from the group consisting of:

(1) a DNA sequence encoding a normal CFTR protein having the amino acid sequence depicted in FIG. 1;

(2) a DNA sequence which hybridizes under stringent conditions to at least 16

contiguous nucleotides of the DNA sequence of (1); and

(3) a DNA sequence complementary to the DNA sequence of (1) or (2), and

(B) providing at least one reagent for detecting the hybridization of the oligonucleotide probe to said normal DNA molecule; or

(ii) assaying for the presence of a mutant CFTR gene by hybridization comprising:

(A) an oligonucleotide probe which specifically binds to a mutant DNA molecule encoding a mutant CFTR polypeptide, wherein the mutant DNA molecule comprises a DNA sequence selected from the group consisting of:

(1) a DNA sequence encoding a mutant CFTR protein having the amino acid sequence depicted in FIG. 1 with a .DELTA.F508 CF mutation as a three base pair deletion of the codon encoding phenylalanine at amino acid position 508 in FIG. 1;

(2) a DNA sequence which hybridizes under stringent conditions to at least 16 contiguous nucleotides of the DNA sequence of (1), said DNA sequence containing said .DELTA.F508 CF mutation; and

(3) a DNA sequence complementary to the DNA sequence of (1) or (2); and

(B) providing at least one reagent for detecting the hybridization of the oligonucleotide probe to said mutant DNA molecule,

wherein the probe and the reagent in (i) and (ii) are each present in amounts effective to perform the hybridization assay.

2. A kit for assaying the presence of a normal CFTR gene by hybridization, comprising:

(a) an oligonucleotide probe which specifically binds to a normal DNA molecule comprising a DNA sequence selected from the group consisting of:

(i) a DNA sequence encoding a normal CFTR protein having the amino acid sequence depicted in FIG. 1;

(ii) a DNA sequence which hybridizes under stringent conditions to at least 16 contiguous nucleotides of the DNA sequence of (i); and

(iii) a DNA sequence complementary to the DNA sequence of (i) or (ii); and

(b) at least one reagent for detecting the hybridization of the oligonucleotide probe to said DNA molecule,

wherein the probe and the reagent are each present in amounts effective to perform the hybridization assay.

3. A kit for assaying the presence of a mutant CFTR gene by hybridization comprising:

(a) an oligonucleotide probe which specifically binds to a mutant DNA molecule

comprising a DNA sequence selected from the group consisting of:

(i) a DNA sequence encoding a mutant CFTR protein having the amino acid sequence depicted in FIG. 1 with a .DELTA.F508 mutation as a three base pair deletion of the codon encoding phenylalanine at amino acid position 508 in FIG. 1;

(ii) a DNA sequence which hybridizes under stringent conditions to at least 16 contiguous nucleotides of the DNA sequence of (i), said DNA sequence containing the .DELTA.F508 CF mutation; and

(iii) a DNA sequence complementary to the DNA sequence of (i) or (ii); and

(b) at least one reagent for detecting the hybridization of the oligonucleotide probe to the DNA molecule,

wherein the probe and the reagent are each present in amounts effective to perform the hybridization assay.

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